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PCA

PROGRESS REPORT I

RESULTS OF EXPERIMENTS TO IMPROVE TECHNIQUES FOR SAMPLING OVERWINTERING SPRUCE BUDWORM POPULATIONS IN OREGON AND WASHINGTON

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
OBJECTIVES	1
STUDY METHODS AND PROCEDURE	2
Design of Experiment	2
Rearing Boxes Tested	2
Selection of Samples	3
Rearing Techniques	4
Supplemental Experiments	4
RESULTS AND DISCUSSION	6
Effect of Box Type	6
Effect of Sampling Location	7
Supplemental Observations	8
RECOMMENDATIONS	8
APPENDIX	10

TABLES AND ILLUSTRATIONS

Plate I.	Characteristics of Sampled Trees	1a
Plate II.	Appearance of Bark at Sampling Points	2a
Plate III.	Collecting and Rearing Techniques	3a
Table 1.	Summary of Larval Emergence	4a
Table 2.	Larval Emergence by Box Type	5a
Table 3.	Larval Emergence by Sampling Location	6a
Graph 1.	Daily Larval Emergence	7a
Graph 2.	Relative Humidity Trends in Rearing Boxes	8a

INTRODUCTION

During the past three years direct control of the spruce budworm (Choristoneura fumiferana Clem.) has been carried out on some 2,128,000 acres of fir forest in Oregon and Washington. An important phase of each season's project has been the sampling and forced rearing of the overwintering larvae in order to determine the status of the infestation on the areas scheduled for treatment.[1]

The type of rearing cage used in the Northwest has been subject to criticism in several respects, and it has been suggested that other types might prove more satisfactory. Also, misleading results from winter population samples collected in 1951 led investigators to believe that perhaps the hibernating larvae are not as abundant in the location that has been used for sampling (the limbs) as other portions of the tree.

With the solution of these problems as an objective, a detailed investigative work plan was prepared and submitted on January 15, 1952 by K. Wright of the Portland Forest Insect Laboratory and A. Lindsten of the Oregon State Board of Forestry. The following report describes the sampling methods used, the rearing techniques employed and the results and application of the findings of the cooperative experiment carried out by the Oregon State Board of Forestry and the Portland Insect Laboratory of the U. S. Bureau of Entomology and Plant Quarantine.

OBJECTIVES

The main objectives of the experiment were to (1) determine which of three types of rearing cages that have been used in various parts of the country is best suited to rearing conditions in this region and (2) to determine which of four known hibernating locations on Douglas-fir trees is the most reliable for yielding a true sample of the overwintering budworm population.

Because there were limitations to the size of experiment that could be undertaken, the original intentions were to test only the first objective at this time, and once the best type of rearing cage had been determined, to undertake the problem of investigating the favorite hibernating location of the budworm. After some deliberation it was decided that at least partial information could be obtained on the second objective at the same time by combining the tests in a randomized block experiment in such a way that the test for box type would not be weakened. This was done and the methods used are described in the following section.

1. Report prepared by H. Haglund - Sampling Overwintering Spruce Budworm Larvae.

STUDY METHODS AND PROCEDURE

Design of Experiment

As mentioned above the experiment was set up in a randomized block manner. The major sampling unit or "block" was the tree and the two categories of "treatment" were the three box types and the four sampling locations on the trees. Three trees were sampled, with 12 collections of rearing material obtained from each tree as follows:

Coeur d'Alene Type Box	New Haven Type Box	Portland Type Box
1 - box lower bole bark	1 - box lower bole bark	1 - box lower bole bark
1 - box mid bole bark	1 - box mid bole bark	1 - box mid bole bark
1 - box upper bole bark	1 - box upper bole bark	1 - box upper bole bark
1 - box limbs	1 - box limbs	1 - box limbs

Total = 12 boxes

By replicating the experiment three times on three trees a total of 36 boxes or observations were obtained.

Rearing Boxes Tested

The three types of rearing boxes tested in the experiment are illustrated in Plate III, figure 4; a further description of the boxes follows:

Coeur d'Alene Box - This box is a common 5-gallon ice cream container measuring nine inches in diameter by nineteen inches in length. The inner surface of the container is heavily waxed making it virtually water-tight. A strip of pasteboard, 1/8" x 2" x 7", was glued to the outer surface of the cover prior to drilling holes for the emergence vials. This was done to provide sufficient support for the vials that the thin cover lacks in itself. This type container or cage is the one used for forcing the emergence of hibernating spruce budworm larvae at the Coeur d'Alene, Idaho, Forest Insect Laboratory.

New Haven Box - This box was developed at the New Haven, Connecticut, Forest Insect Laboratory, specifically for collection of budworm larvae as they issue from hibernation. The box is $5\frac{1}{2}$" x 11" x 15" in size and is lined throughout with waxed "butcher" paper. The holes in which the emergence vials are inserted are backed-up with a piece of 5/8" plywood.

Portland Box - This type of cage or box has been used for rearing overwintering larvae in connection with the Oregon and Washington control projects for the last three years, and is a common pasteboard carton measuring 13" x 13" x 24". The boxes have no inner lining and are unaltered except for three holes drilled in one end of the box to hold the collection vials.

Selection of Samples

Samples were collected on January 29, and 30, 1952 from three Douglas-fir trees located in the Waterman Flat area of north central Oregon near Mitchell. The sample trees were located about one-half mile apart in a moderately to heavily defoliated stand of predominantly Douglas-fir timber. Budworm egg masses were observed to be numerous on the selected trees. The trees were felled and the rearing material collected and sealed in the boxes in the field so as to minimize damage to the larvae while in transit to the laboratory.

Bark surface from four locations on each of the three trees was sampled; three of the samples were taken from the main bole at breast height, mid bole and upper bole, with the fourth sample consisting of the basal portion of limbs at midcrown. Description as to the nature of the sample trees and the points of sampling on each tree are given in the following table.

Tree No.	Species	D.B.H.	Age	Total Height	Crown Length	Height Upper Bole Sample
1	Douglas-fir	15.1	56	85'	72'	52'
2	Douglas-fir	14	60	60'	44'	33'
3	Douglas-fir	16	58	93'	70'	60'

Bole Samples - To secure the bole samples it was necessary to cut 14-inch blocks from each of the sampling positions. The breast height or lower bole sample was taken at 4½ feet above the ground, the mid bole sample at an arbitrary point one-half the distance between the ground line and the location where rough bark gives way to smooth bark, and the upper bole sample at the aforementioned point where rough bark gives way to smooth bark. Photographs showing the comparative roughness of bark at these three points on sample tree number 2 are shown in Plate II, figures 1, 2 and 3.

After each block section was removed from the tree its circumference was divided into three equal segments from which three tangential sections containing both sapwood and bark were removed, using a maul and wedges (see Plate III, figures 1 and 2). At least 200 square inches of bark surface were included with each of the sections. These were then assigned at random to the three types of boxes in such a way that the three sides of the trees sampled were equally represented in each type box.

Limb Samples - Nine limbs, approximately 2½ inches in diameter at the base were removed from midcrown of each of the trees. It was noted that the bark on the limbs of all trees in the sample area were relatively smooth. Beginning at the base of the limbs, three 14-inch sections were cut from each one, numbering each consecutively 1, 2, and 3 as it was cut. In this manner 27 limb sections were cut from the 9 branches. When assigning the limbs to the 3 types of boxes, care was taken that each box contained three number 1 cuts, three number 2 cuts and three number 3 cuts.

Rearing Techniques

The material was returned to the Oregon State Board of Forestry headquarters at Salem immediately after collection, and was placed in rearing February 1. To comply with the requirements of the randomized block experiment the 36 boxes were arranged in three tiers, with the material from tree number one occupying the upper tier, tree number two the middle tier and tree number three the bottom tier as shown in Plate III, figure 5. The 12 boxes in each tier (four of each type) were randomized as to position to offset any differences in light, temperature or other influencing factors that might exist.

A string of nine 100-watt incandescent light bulbs was hung approximately 30 inches from the front of the middle row of boxes, and kept burning continuously night and day. Temperatures in the rearing room were kept between 60 and 70 degrees Fahrenheit.

The relative humidity in the boxes was maintained as near 65 percent as possible through use of a simple dial-type humidiguide installed in the end of one of each of the three types of boxes. It was found necessary to ventilate the Coeur d'Alene boxes to reduce the relative humidity; this was accomplished by cutting 1" x 1" holes in each end of the boxes and covering them with a very fine wire screen. The screened holes were opened and closed with masking tape as adjustments in humidity were needed. The New Haven boxes remained very close to the optimum humidity, while the unlined Portland boxes required spraying daily with an atomizer to increase the moisture. Larvae were collected daily when emergence began and individual records were kept for each of the 36 boxes.

Supplemental Experiments

As sidelights to the main experiment, small-scale tests were made to determine (1) the presence or absence of hibernating budworm larvae on foliage and in the duff, and (2) the relative humidity that could be expected to be maintained throughout the course of a normal rearing period in the three types of boxes if no humidity control was attempted.

The additional tests on hibernation location consisted merely of boxing quantities of foliage and duff. Using the Coeur d'Alene type box only, one container of foliage was collected from each of the three trees sampled in the main experiment; a single container of duff from around the base of the second tree sampled was collected. These collections were placed in rearing along with the other material.

Research conducted in the Northeastern States and Canada has shown the importance of maintaining the relative humidity at least reasonably near an optimum range in the cages. Accordingly, as an additional evaluation of the three box types tested in the randomized block experiment, one box of each

type, containing some 200 square inches of bark, was fitted with a sensitive hair-fillament hygrothermograph and allowed to run its course without any provisions being made for raising or lowering the relative humidity. Through windows installed in the sides of the boxes, humidity readings were taken from the instrument three times daily.

RESULTS AND DISCUSSION

The total number of budworm larvae obtained from the 36 individual rearings is shown in table 1. Because the volume of bark surface caged in each box varied it was necessary to reduce all emergence to a common base; emergence on an arbitrary 100 square inches of bark basis was therefore computed and is also shown in table 1. Tables 2 and 3 show, respectively, breakdowns of the larval emergence according to type of rearing box and sampling location. Graph 1 shows the nature of the daily emergence of the larvae according to box type, and graph 2 depicts the relative humidity maintained in the three boxes in which the hair-fillament hygrothermographs were sealed.

In addition to the comparison of arithmetic sums and averages made in the above tables a statistical analysis of the data appropriate to the randomized block experimental design was made. Results of both methods of analysis are discussed below.

Effect of Box Type

As shown in table 2 little difference between the three box types was found. Although the Portland type box surprisingly yielded a somewhat higher number of larvae per 100 square inches of bark caged (20.5) as compared to the Coeur d'Alene and New Haven boxes (17.4 and 16.9 respectively) an analysis of variance of the data showed no significant difference between them.

Perhaps the strongest conclusion that can be drawn from this phase of the experiment is that the three types of boxes are equally suitable, within practical limits, for rearing providing the humidity within the boxes is kept somewhere near the optimum range. Although the exact effect of humidity on the emergence was not determined in the tests, it is felt that "adequate" moisture is a highly important factor. From the hygrothermograph readings it was learned that the unlined Portland type boxes lost moisture rapidly; it is a matter of conjecture as to how many larvae they would have produced if moisture had not been added.

Some feeling previously existed that the large size of the Portland box caused the larvae to travel excessively long distances before finding their way into the collection vials; from the results this factor apparently is not a limiting one. The smooth-surfaced Coeur d'Alene box is almost entirely free of seams, corners, and other hiding places found in the Portland and New Haven boxes that might well have considerable effect on the larvae finding their way into the collection vials. There are two other features of the Coeur d'Alene type box that make it a desirable cage for rearing overwintering larvae -- (1) to close the box all that is needed is to put on the cover and seal with a single strip of masking tape, and (2) the boxes take up a minimum of space

in the laboratory. In contrast, the New Haven boxes, although of a convenient size, are costly and difficult to construct and require taping to seal them; the Portland boxes are extremely bulky and also require that considerable time be spent taping them shut.

The only shortcoming that can be seen at this time to the Coeur d'Alene boxes is the possibility of them becoming too humid, causing mold to form on the rearing material and thus inhibiting emergence of larvae from their hibernacula. Excessive humidity was encountered with the cages containing bole sections, which consisted of considerable amounts of sapwood in addition to the bark. However, by periodically ventilating the boxes through the small screen openings mold was kept from forming on the contents. It was noted that when moisture began to accumulate in the emergence vials the hygrothermographs indicated relative humidities of approximately 80 per cent; steps were immediately taken to ventilate the boxes when this condition existed.

Effect of Sampling Location

The outstanding feature of this phase of the experiment to determine the best sampling location among limbs and the main bole samples at breast height, mid bole and upper bole was the very small number of larvae obtained from the limbs as compared with the other locations. A summary of these results is shown in table 3.

From inspection of the table it is found that the mid bole sample produced the greatest number of larvae per 100 square inches of bark surface (41.8) as compared with the samples at breast height (31.7), upper bole (31.3) and the limbs (1.3). From the analysis of variance computations, however, it was determined that among the three locations producing relatively good results, none is significantly better than another, although each is obviously better than the limbs. The reason for the failure of one of the bole locations to prove significantly better than another was the wide variance (interaction) found between the three trees and the number of larvae produced from the three different sampling locations. That is, on trees number 1 and 3 the upper bole sample yielded the greatest number of larvae, while with tree number 2 the breast height sample was the best producer. The mid bole sample, on the other hand, "averaged" better than the other locations.

From the foregoing discussion the following conclusions are drawn.

1. The limbs did not produce a representative sample of the larval population on the sample trees. This outcome was probably largely traceable to the relatively smooth limbs found on the majority of the Douglas-fir trees in the Waterman Flat area. The small recovery of larvae largely explains the misleading rearing results obtained during the winter of 1951, when limbs were sampled, that resulted in elimination of the Waterman area from control consideration.

2. Due to the strong inter-tree variation in sampling location encountered in the test no one of the three sampling locations at breast height, mid bole, and upper bole is regarded as superior to another; all were far superior to the limbs.

Supplemental Observations

As sidelights to the main experiment it was pointed out in the preceding main section that small-scale investigations were made of (1) additional sampling locations for hibernating larvae and (2) the relative humidity maintained in the three types of cages throughout the rearing period.

Only one budworm larvae was obtained from the three boxes of foliage that were collected from the sample trees. No larvae were reared from the caged duff collected from the base of sample tree number two. These tests substantiated previous findings that the budworm does not hibernate in as large numbers on foliage in the Northwest as it does in the northeastern states, and also gave reason to believe that the larvae do not hibernate in the duff.

Results of the relative humidity tests were of considerable interest and are shown in graph 2. Each daily observation represents the average of three readings taken from the hygrothermographs. As might be expected the virtually water-tight Coeur d'Alene boxes maintained very high humidities throughout the 22 days the readings were taken. This condition resulted in formation of considerable mold on the caged bole section.

The New Haven box maintained a relatively constant humidity, no doubt due to the waxed paper lining, which was some 5 to 8 percent over the regarded optimum of 85. The relative humidity in the Portland box, as in the other types, rose sharply immediately after sealing; contrary to the others, however, the humidity in this box dropped off sharply after a few days and for the balance of the test period remained only slightly higher than the humidity in the laboratory. Apparently the sides of the unlined box are highly hygroscopic, resulting in rapid adjustment of the moisture condition in the box to that of the room.

RECOMMENDATIONS

From the results of the experiment certain conclusions can be drawn. Also, as with most experiments, the results suggest further investigations that should be undertaken to solve other phases of the overall problem. These items are summarized as recommendations and are given below.

1. The Coeur d'Alene-type box (a 5-gallon ice cream container) serves as the best rearing cage in this region. The only qualifications to its use is that provision be made to ventilate the boxes and additional reinforcement to the top.

2. In areas east of the Cascades where the limbs on the trees are smooth, or relatively so, samples for rearing should be taken either from breast height, mid bole, or upper bole.

3. Follow-up tests on the above three recommended sampling locations should be undertaken to further investigate the inter-tree variation of hibernation location.

4. When bole sections are used as rearing material, 200 square inches of bark surface is adequate for each collection when sampling moderate to heavy populations.

5. The experiment just completed should be duplicated, using the Coeur d'Alene box, in stands where rough limbs are found. Limbs are a highly desirable sampling medium because they can be secured without damaging the trees; collecting them should not be abandoned on all areas until more information on this point is obtained.

6. Experiments similar to this one, using one type of box only, should be carried out in western Oregon stands, where characteristics of the trees to be sampled are much different than those east of the Cascades.

APPENDIX

PLATE I

Characteristics of Sampled Trees

Fig. 1. Typical Waterman Flat stand used for the experimental sampling. Note overstory of pine with understory of Douglas-fir and white fir.

Fig. 2. Appearance of 1951 defoliation on sample tree number 1 as photographed in January 1952. Severity of damage is typical of that found in the Waterman area.

Fig. 3. Nature of the bark surface near breast height on 50 to 60 year old Douglas-fir from which overwintering spruce budworm larvae were reared.

PLATE II

Appearance of Bark at Sampling Points (From Sample Tree Number 3)

Fig. 1. Upper bole

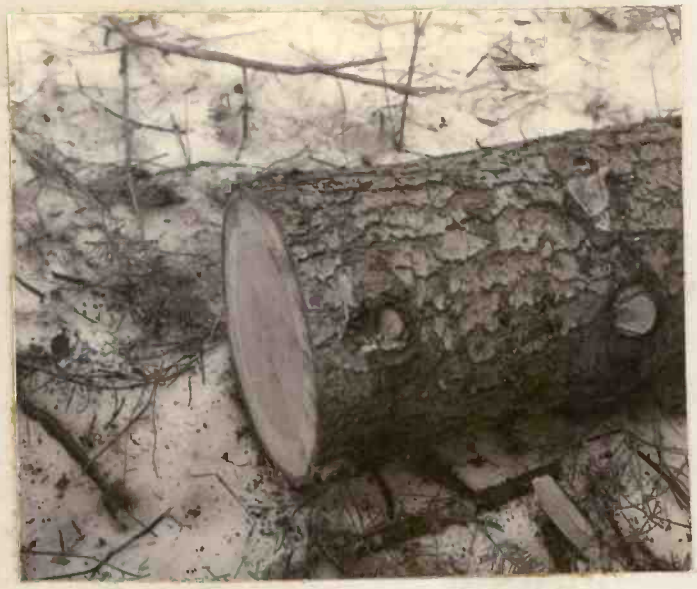


Fig. 2. Mid bole.

Fig. 3. Breast height.

Fig. 4. Limbs.

Collecting and Rearing Techniques

Fig. 1. Section removed from main bole of sample tree.

Fig. 2. Removal of tangential section of bole sample containing approximately 200 square inches of bark surface.

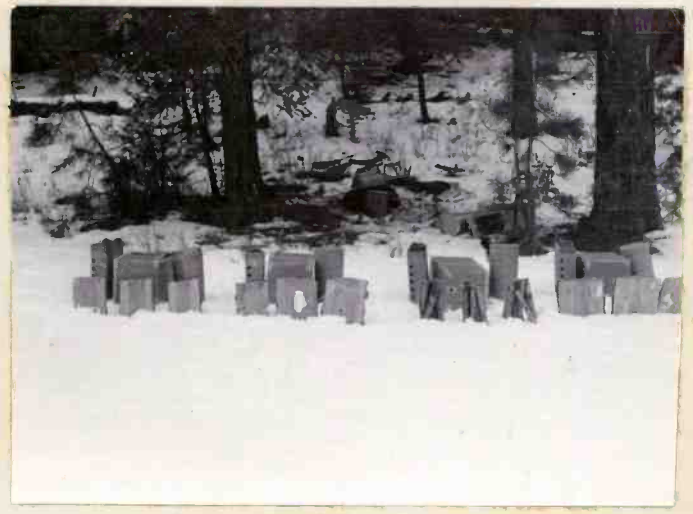


Fig. 3. Complete set of rearing material from one sample tree.

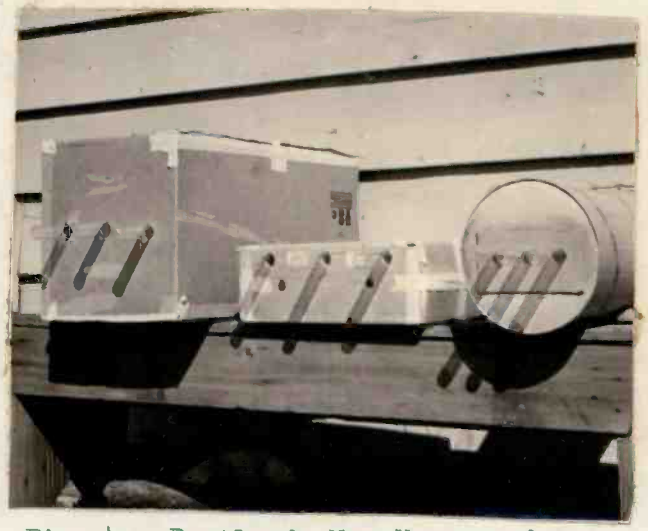


Fig. 4. Portland, New Haven and Coeur d'Alene experimental rearing boxes (l. to r.).

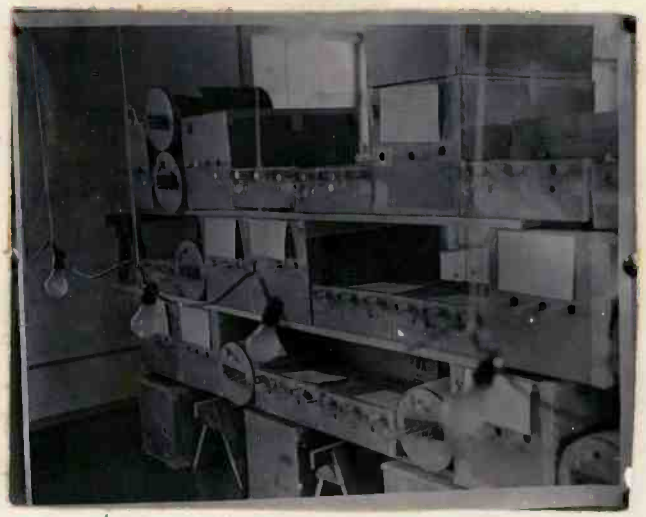


Fig. 5. Laboratory rearing setup used for forcing the emergence of overwintering spruce budworm larvae.

Table 1 Total Emergence of Hibernating Spruce Budworm Larvae Obtained from 36 Samples of Limb and Bark Material Collected from 3 Douglas-fir Trees in the Ochoco Area of North-Central Oregon

Tree Number and Location sampled	TYPE OF REARING BOX											
	Coeur d'Alene			New Haven			Portland			Total		
	Sq. in. Bark	Total Emerg.	Emerg. per 100 sq. in.	Sq. in. Bark	Total Emerg.	Emerg. per 100 sq. in.	Sq. in. Bark	Total Emerg.	Emerg. per 100 sq.in.	Sq. in. Bark	Total Emerg.	Emerg. per 100 sq.in.
Tree No. 1												
Limbs	637	28	4.4	664	16	2.4	659	11	1.7	1960	55	2.8
Breast Height	219	29	13.2	201	8	4.0	218	38	17.4	638	75	11.7
Mid Bole	210	128	60.9	223	67	30.0	201	77	38.3	634	272	42.9
Upper Bole	210	176	83.8	227	98	43.2	210	107	50.9	647	381	58.9
Subtotal	1276	361	28.3	1315	189	14.4	1288	233	18.1	3879	783	20.2
Tree No. 2												
Limbs	594	0	0.0	580	5	0.9	550	1	0.2	1724	6	0.3
Breast Height	208	89	42.8	210	145	69.0	223	317	142.6	641	551	86.0
Mid Bole	217	107	49.3	244	243	99.6	221	164	74.2	682	514	75.3
Upper Bole	211	23	10.9	209	26	12.4	216	36	16.7	636	85	13.4
Subtotal	1230	219	17.8	1243	419	33.7	1210	518	42.8	3683	1156	31.4
Tree No. 3												
Limbs	654	1	0.2	665	0	0.0	661	1	0.2	1980	2	0.1
Breast Height	210	1	0.5	239	1	0.4	210	0	0.0	659	2	0.3
Mid Bole	206	6	2.9	203	14	6.9	206	1	0.5	615	21	3.4
Upper Bole	205	71	34.6	205	33	16.1	210	25	11.9	620	129	20.8
Subtotal	1275	79	6.2	1312	48	3.7	1287	27	2.1	3874	154	4.0
TOTAL	3781	659	17.4	3870	656	16.9	3785	778	20.5	11436	2093	18.3

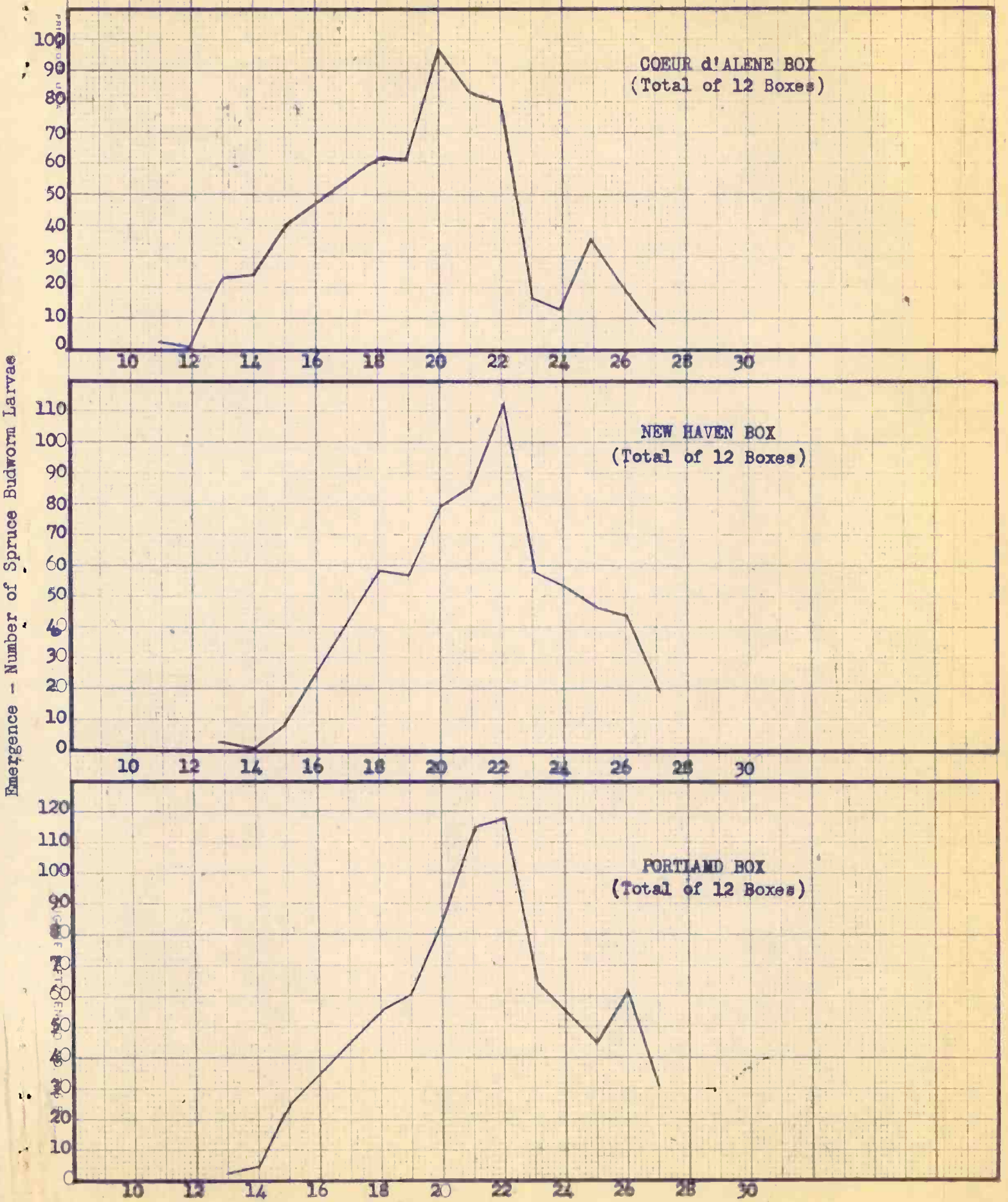
Table 2 Summary of Emergence of Overwintering Spruce Budworm Larvae Obtained From Three Types of Rearing Boxes

Tree Number and Location on Tree	Box Type (Emergence per 100 square Inches Bark Surface)			
	Coeur d'Alene	New Haven	Portland	Average per 100 square inch
Tree No. 1				
Limbs	4.4	2.4	1.7	2.8
Breast Height	13.2	4.0	17.4	11.7
Mid Bole	60.9	30.0	38.3	42.9
Upper Bole	83.8	43.2	50.9	58.9
Average per 100 sq. in.	28.3	14.4	18.1	20.2
Tree No. 2				
Limbs	0.0	0.9	0.2	0.3
Breast Height	42.8	69.0	142.6	86.0
Mid Bole	49.3	99.6	74.2	75.3
Upper Bole	10.9	12.4	16.7	13.4
Average per 100 sq. in.	17.8	33.7	42.8	31.4
Tree No. 3				
Limbs	0.2	0.0	0.2	0.1
Breast Height	0.5	0.4	0.0	0.3
Mid Bole	2.9	6.9	0.5	3.4
Upper Bole	34.6	16.1	11 9	20.8
Average per 100 sq. in.	6.2	3.7	2.1	4.0
SUMMARY:				
Ave./100 sq. in. Bark Surface	17.4	16.9	20.5	18.3

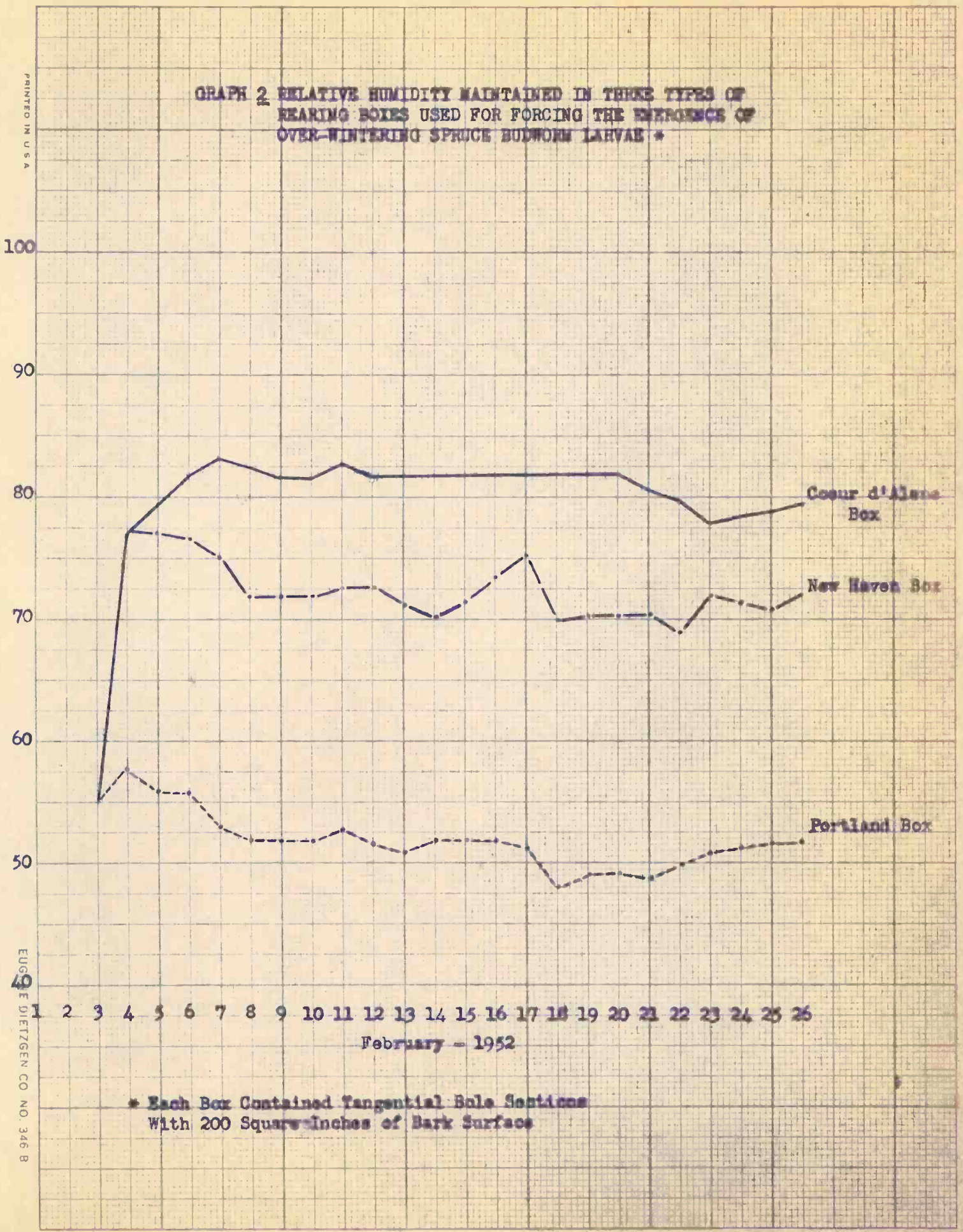
Table 3 Summary of Emergence of Overwintering Spruce Budworm Larvae Obtained from Four Sampling Locations On Each of Three Douglas-fir Trees

Tree Number and Box Type	Location (Emergence Per 100 Square inches of Bark Surface)				
	Limbs	Breast Height	Mid bole	Upper bole	Average per 100 sq. in.
Tree No. 1					
Coeur d'Alene	4.4	13.2	60.9	83.8	28.3
New Haven	2.4	4.0	30.0	43.2	14.4
Portland	1.7	17.4	38.3	50.9	18.1
Average per 100 sq.in.	2.8	11.7	42.9	58.9	20.2
Tree No. 2					
Coeur d'Alene	0.0	42.8	40.3	10.9	17.8
New Haven	0.9	69.0	99.6	12.4	33.7
Portland	0.2	142.6	74.2	16.7	42.8
Average per 100 sq.in.	0.3	86.0	75.3	13.4	31.4
Tree No. 3					
Coeur d'Alene	0.2	0.5	2.9	34.6	6.2
New Haven	0.0	0.4	6.9	16.1	3.7
Portland	0.2	0.0	0.5	11.9	2.1
Average per 100 sq.in.	0.1	0.3	33.4	20.8	4.0
SUMMARY:					
Ave/100 sq. in. bark surface	1.1	32.4	41.8	31.3	18.3

GRAPH 1 TRENDS OF EMERGENCE OF SPRUCE BUDWORM LARVAE FROM INFESTED LIMBS AND BARK CAGED IN THREE TYPES OF REARING BOXES
(Rearing Material Caged 2-1-52)


COEUR d'ALENE BOX
(Total of 12 Boxes)
NEW HAVEN BOX
(Total of 12 Boxes)
PORTLAND BOX
(Total of 12 Boxes)
Emergence - Number of Spruce Budworm Larvae
10 12 14 16 18 20 22 24 26 28 30
February - 1952

GRAPH 2 RELATIVE HUMIDITY MAINTAINED IN THREE TYPES OF REARING BOXES USED FOR FORCING THE EMERGENCE OF OVER-WINTERING SPRUCE BUDWORM LARVAE *



* Each Box Contained Tangential Bole Sections With 200 Square Inches of Bark Surface

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